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Survivin Gene Polymorphism Association with Tongue Squamous Cell Carcinoma

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Subject: Survivin expression is correlated with tumor aggressiveness and severity in head and neck carcinoma. A polymorphism at position −31 (G/C) (rs 9904341) has been associated with cancer risk in several studies. We evaluated the correlation of this polymorphism with clinical manifestation of patients with tongue squamous cell carcinoma (SCC) in an Irananian population. *Methods:* Paraffin-embedded tissue sections from patients with tongue SCC ($n=91$) were evaluated for association between the *survivin* −31 (G/C) polymorphism and tumor staging, pathological grade, lymph node metastasis, tumor size, and recurrence of tumor. *Results:* There was a significant increase of presence of allele C in patients who were at stages III and IV compared to patients with lower stages [GC+CC vs. GG, $p=0.025$, odds ratio [OR] 2.76, 95% confidence interval [CI] [1.03–7.4]). In addition, presence of allele C was significantly decreased in patients with T1 tumor size compared to patients with larger tumor size ($p=0.03$, OR 0.6, 95% CI [0.2–2.03]). *Conclusion:* Presence of the C allele was significantly associated with tumor stage and size; therefore, survivin might be an important marker in the prognosis of tongue SCC that requires further investigation.

Introduction

Carcinoma of the oral cavity comprises ~14% of head and neck malignancies with tongue carcinoma as the most common type of oral carcinoma presenting as the squamous cell carcinoma (SCC) type in the majority of cases (86%) and causing high morbidity (Funk *et al.*, 2002).

The prevalence of tongue SCC is increased in the world, and despite, aggressive therapeutic strategies have remained ubiquitous with high morbidity and mortality rate (Funk *et al.*, 2002). The 5-year survival rates in the best cases are <60% (Funk *et al.*, 2002).

In a study by Schantz *et al.* (2002), the prevalence of tongue carcinoma has been increased up to 60% in patients younger than 40 years of age, between 1973 and 1984, and has remained the same since then, while the prevalence of other types of oral cavity carcinoma did not change at the same period. Despite this increase, the 5-year determinant survival of these patients was better than that for older individuals (Schantz *et al.*, 2002).

The exact etiology for the increased prevalence of disease is not clear, and some factors, including drug addiction, smoking, and HPV infection, were suggested (Woods *et al.*, 1993).

Overall, two main mechanisms have been proposed for the pathogenesis of head and neck carcinomas: environmental carcinogens and genetic susceptibility (Thomas *et al.*, 2005; Ferrari *et al.*, 2009).

Genetic mutations and abnormal expression of molecular markers have been reported in most cases (Thompson, 1995; Forastiere *et al.*, 2001; Belbin *et al.*, 2008; Ferrari *et al.*, 2009). There are small, rather controversial, data regarding the molecular mechanisms involved in pathogenesis of tongue carcinoma (Thompson, 1995). Recent evaluations are mainly based on clinical examination and histopathology results. More information on molecular and genetic mechanisms involved will be valuable for the assessment of prognosis and disease outcome.

Apoptosis and unbalanced cell proliferation play a critical role in development of cancers (Melet *et al.*, 2008). The human inhibitor of apoptosis protein (IAP) family consists of eight members of baculoviral repeat containing BIRC 1 to BIRC 8 (Johnson and Howerth, 2004; Knauer *et al.*, 2007). High expression of *survivin* (*BIRC 5*) and XIAP (BIRC 4) is critical for apoptosis suppression in human solid tumors (Krepela *et al.*, 2009). Survivin has unique propriety because of its bifunctional role as a protein that exhibits cell cycle

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regulation and inhibition of apoptosis (Knauer *et al.*, 2007). It is expressed in the G2/M phase of cell cycle to support the rapidly dividing cell machinery (Kawata *et al.*, 2011). Importantly, survivin is generally expressed in embryonic tissues; therefore, lack of survivin function results in disorganized and embryonal death (Borges *et al.*, 2010; Upadhyay *et al.*, 2011).

Recently, some investigators have found evidence of survivin expression in normal adult cells; therefore, it has been speculated that it may also have a role in normal cellular function (Yang *et al.*, 2009b; Bayram *et al.*, 2011).

The *survivin* gene in humans spans 14.7 kb located at the telomeric region of chromosome 17q25 (Ambrosini *et al.*, 1998). Several single-nucleotide polymorphisms (SNPs) were identified within the promoter region of the *survivin* gene. A polymorphism at position −31 that involves the substitution of G for C (rs 9904341) has been the most documented in previous reports (Ma *et al.*, 2011). This polymorphism is located at the cell cycle-dependent elements and cell cycle homology region repressor-binding motif of the promoter (Ma *et al.*, 2011), and this mutation (G/C) seems to be correlated with increased expression of survivin at both transcription and translation levels (Lo and Farina, 2005; Ma *et al.*, 2011). Also, many other *survivin* promoter polymorphisms, including −644T>C, −625G>C, and −241T>C, have been reported to be in linkage disequilibrium with −31G>C (Yang *et al.*, 2009b).

Several case–control studies have examined the association between −31G/C polymorphism and cancer risk (Srivastava *et al.*, 2012), including nasopharyngeal carcinoma (Yang *et al.*, 2009a), esophaseal cancer (Yang *et al.*, 2009b), gastric cancer (Yang *et al.*, 2009a; Borges *et al.*, 2010), hepatocellular carcinoma (Bayram *et al.*, 2011), pancreatic cancer (Theodoropoulos *et al.*, 2010), and urothelial carcinoma (Wang *et al.*, 2009). Immunohistochemical staining for survivin expression in tissue samples of thyroid carcinoma has shown the increased expression of survivin in thyroid carcinoma (Haghpanah *et al.*, 2009). In a study on 110 patients with oral SCC, survivin expression was observed in 82.7% of patients, and all cases with metastatic carcinoma were positive for survivin expression. Reduced survivin expression was associated with long-term survival (Lo *et al.*, 2003), indicating the relationship between survival and more aggressive tumor behavior and disease severity (Lo *et al.*, 2003). According to the critical role of survivin in carcinogenesis with prognostic and its therapeutic implications (Dai *et al.*, 2010), we evaluated the correlation of this polymorphism with pathological finding in tongue SCC in an Irananian population.

Materials and Methods

Study population

Formalin-fixed, paraffin-embedded tissue sections were recruited from the archive of pathology department, Amir-Alam hospital, after being examined by an expert pathologist.

Staging for tongue SCC was defined according to the American Joint Committee on Cancer (AJCC) and follows TNM staging. The case group consisted of patients with tongue SCC ($n=91$). The study was approved by the Ethics committee of Tehran University. Informed consents were obtained from all of the patients in the study.

DNA extraction and genotyping

DNA was extracted from paraffin-embedded tissue sections as described previously (Zahedi *et al.*, 2012).

The 151-bp DNA fragment containing the −31 polymorphic site amplified using pairs of primers as follows:

5′-AAGAGGGCGTGCGCTCCCGACA-3′ and

5′-GAGATGCGGTGgTCCTTGAGAAA-3′.

The polymerase chain reaction (PCR) was performed in a total volume of 20 μL containing 2 μL 10× PCR buffer (Fermentas), 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.375 μM of each primer, 200 ng genomic DNA, and 1 U of *Taq* DNA polymerase (Fermentas).

The amplification conditions were as follows: denaturation at 95°C for 10 min; five cycles of 95°C for 45 s; and 72°C for 60 s for primer annealing, followed by 30 cycles of 94°C for 45 s, 62°C for 45 s, and 72°C for 45 s, with a final elongation at 72°C for 10 min. The PCR product size was 151 bp.

Eight microliters of PCR product was digested with 5 u *Msp*I at 37°C overnight. Digested products yielding a 151-bp uncut fragment for the GG genotype, two fragments of 61 and 90 bp for the CC genotype, and three fragments of 151, 90, and 61 bp for the CG genotype were visualized on a 4% agarose gel stained with ethidium bromide.

Statistical analysis

Strength of association between different groups and alleles or genotypes of the survivin gene polymorphism was estimated using odds ratios (ORs) and 95% confidence intervals (CIs). Levels of significance were determined using contingency tables by either Chi-square or Fisher exact analysis.

All analyses were carried out using STATA version 8. $p \leq 0.05$ was considered a significant statistical difference.

Results

The mean age of patients was 55.7 ± 16.4 years, and women accounted for 49% and men 51% of the study population. Among our patients, 86% accounted for well-differentiated tumors. None of the patients presented with metastasis while nodal involvement was reported in 66% of patients. The clinical characteristics of patients are given in Table 1.

Survivin *gene −31 G/C polymorphism allele and genotype frequencies in patients with tongue SCC*

The allele and genotype frequencies of the survivin gene −31G/C polymorphism were determined in patients with tongue SCC. When we compared the frequency of the survivin gene polymorphism in patients with stages III and IV to patients with stages I and II, we observed that the frequency of the GC or CC genotype was significantly higher in patients with stages III and IV [GC+CC vs. GG, $p=0.025$, OR 2.76, 95% CI (1.03–7.4)] (Table 2). In addition, presence of allele C was significantly decreased in patients with T1 tumor size (23%) compared to patients with larger tumor size (48%) ($p=0.03$, OR 0.6, 95% CI [0.2–2.03]) (data not shown).

Although there was an increase in the frequency of the GC or CC genotype in patients with lymph node involvement (55%) compared to patients without lymph node involvement (33%), it did not reach a significant level [($p=0.05$, OR 2.4, 95% CI (0.8–6.6)] (data not shown).

Table 1. The Characteristics of Patients with Tongue Squamous Cell Carcinoma

Carcinoma	n = *91*
Tumor size	
T1	27 (30%)
T2	41 (45%)
T3	11 (12%)
T4a	12 (13%)
Nodal involvement	
N0	60 (66%)
N1	15 (16%)
N2	15 (16%)
N3	1 (1%)
Metastasis	
M0	91 (100%)
M1	0 (0%)
Stage	
I	24 (26%)
II	26 (29%)
III	17 (19%)
IVa	23 (25%)
IVb	1 (1%)
IVc	0 (0%)
Grade	
Well differentiated	78 (86%)
Moderately differentiated	11 (12%)
Poorly differentiated	2 (2%)

There was no significant association between the survivin gene polymorphism and pathological grade in our population.

Discussions

Our results suggest that the *survivin* C −31G polymorphism was associated with stage of disease in our patients with tongue SCC.

The increased expression of survivin in oral SCC and epithelial dysplasia has been reported, indicating its role in tumor aggressiveness and prognosis (Lin *et al.*, 2005; Bang *et al.*, 2008; De Maria *et al.*, 2009). In another study, an association between survivin expression and decreased survival rate in patients with oral SCC was observed and was found as a marker for response to radiotherapy (Freier *et al.*, 2007). Previous studies show that survivin expression is associated with higher grade, larger tumor size, lymph node metastasis, and recurrence in most cases (Lo *et al.*, 2001; Freier *et al.*, 2007; Bang *et al.*, 2008).

The role of anti-survivin antibodies in treatment of patients with head and neck carcinoma has been studied by Myomi *et al.*, and an important role of survivin as a carcinogenic antigen was shown (Eto *et al.*, 2007). In a study on laryngeal SCC, improvement was observed in patients treated with Silibinin, which decreased survivin expression (Bang *et al.*, 2008).

In basal cell carcinoma of the larynx, survivin expression was related to recurrence and cases with poor prognosis requiring closer follow-up and more aggressive treatment (Marioni *et al.*, 2006).

Functional studies have previously investigated the effect of the −31G/C polymorphism using a luciferase assay, indicating that the G allele decreased promoter activity compared with the C allele in Hela and CHO cells (Jang *et al.*, 2008). Allele C was shown to be correlated with overexpression of survivin at both mRNA and protein levels and also with cell cycle-dependent transcription in various cancer cell lines (Gazouli *et al.*, 2009). Our finding is in keeping with previous studies showing the C allele as a risk of developing various carcinomas. We have recently studied the association between this polymorphism and papillary thyroid carcinoma (PTC) and found that this polymorphism was a predisposing factor in PTC, and a more significant increase in the frequency of allele C was observed in patients with profound manifestations, including lymph node involvement, vascular involvement, and multifocality (Yazdani *et al.*, 2012). Also, we observed strong association between this polymorphism and endometrial cancer (Zahedi *et al.*, 2012). Our finding highlights the importance of survivin in pathogenesis of tongue SCC, which might be useful in therapeutic strategies and also have diagnostic implications.

We did not find an association between the *survivin* gene polymorphism and pathological grading in our population, which might be due to the small number of patients in poorly differentiated and moderately differentiated groups. More studies on a larger number of samples and on other groups of patients with benign lesions of the oral cavity are required to further confirm the results we have observed in this study.

Table 2. *Survivin* −31 G/C Polymorphism Allele and Genotype Frequencies in Patients with Tongue Squamous Cell Carcinoma at Different Stages

	Stage			
Survivin	*I*	*II*	*III*	*IV*
Total	19	25	17	24
Genotype				
GG	14 (74%)	17 (68%)	6 (35%)	13 (54%)
GC	4 (21%)	8 (32%)	10 (59%)	8 (33%)
CC	1 (5%)	0 (0%)	1 (6%)	3 (13%)
Allele				
G	32 (84%)	42 (84%)	22 (65%)	34 (71%)
C	6 (16%)	8 (16%)	12 (35%)	14 (29%)

Comparison for frequency of *survivin* gene polymorphism between patients with stages III and IV and patients with stages I and II (GC+CC vs. GG, $p=0.025$, OR 2.76, 95% CI [1.03–7.4]).

CI, confidence interval; OR, odds ratio.

Author Disclosure Statement

No competing financial interests exist.

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